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☐ 1. Document ID: AU 200012030 A, WO 200022110 A2

L4: Entry 1 of 1 File: DWPI May 1, 2000

DERWENT-ACC-NO: 2000-317970
DERWENT-WEEK: 200036

TITLE: Targeting degradation of polypeptide useful for treating cancer and other proliferative disorders, involves conjugating polypeptide with ubiquitin protein ligase or inhibiting ubiquitination using organic compound

INVENTOR: HOWLEY, P; ZHOU, P

PRIORITY-DATA: 1998US-0103787 (October 9, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200012030 A	May 1, 2000	N/A	000	C12N015/00
WO 200022110 A2	April 20, 2000	E	185	C12N015/00

INT-CL (IPC): C07K 14/00; C12N 5/10; C12N 15/00; C12N 15/12; C12N 15/37; C12N 15/52; C12N 15/62

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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Terms	Documents
l2 and l3	1

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L6: Entry 3 of 6 File: USPT Dec 26, 2000

US-PAT-NO: 6165731
DOCUMENT-IDENTIFIER: US 6165731 A

TITLE: Assay for the ubiquitination-promoting activity of human proteins

DATE-ISSUED: December 26, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Deshaies; Raymond	Claremont	CA	N/A	N/A
Lyapina; Svetlana	South Pasadena	CA	N/A	N/A
Correll; Craig C.	Pasadena	CA	N/A	N/A

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
California Institute of Technology	Pasadena	CA	N/A	N/A	02

APPL-NO: 9/ 235572
DATE FILED: January 22, 1999

PARENT-CASE:
CROSS REFERENCE TO RELATED APPLICATIONS This application claims the benefit of the U.S. Provisional Application Nos. 60/072,209, filed on Jan. 22, 1998 and Ser. No. 60/083,447, filed Apr. 29, 1998 which are incorporated herein by reference.

INT-CL: [7] G01N 33/53, G01N 33/573, C12P 13/18, A61K 39/395, C07K 16/00
US-CL-ISSUED: 435/7.1; 435/6, 435/7.4, 435/7.9, 435/193, 435/252.3, 424/141.1, 536/23.2, 536/26.31, 536/26.32, 536/23.5 , 530/387.9
US-CL-CURRENT: 435/7.1; 424/141.1, 435/193, 435/252.3, 435/6, 435/7.4, 435/7.9, 530/387.9, 536/23.2, 536/23.5
FIELD-OF-SEARCH: 435/7.1, 435/7.4, 435/7.9, 435/6, 435/193, 435/252.3, 424/141.1, 536/23.2, 536/24.31-32, 536/23.5, 530/387.9

PRIOR-ART-DISCLOSED:

OTHER PUBLICATIONS

Renny Feldman et al; A Complex of CDC4P, SKP1PAND CDC53P/Cullin Catalyzes Ubiquitination of the Phosphorylated CDK Inhibitor SIC1P; Cell, 91; 221-230, 1997.

ART-UNIT: 165
PRIMARY-EXAMINER: Minnifield; Nita
ASSISTANT-EXAMINER: Baskar; Padma
ATTY-AGENT-FIRM: Gray Cary Ware & Freidenrich LLP Haile; Lisa A.

ABSTRACT:

A method is provided for identifying an compound that affects an activity of a polypeptide subunit of a SCF complex. The method includes contacting a sample comprising a chimeric SCF complex assembled from subunits derived from Saccharomyces cerevisiae or human and another species and a CDC34p polypeptide with the compound under conditions that allow the components to interact, and adding to these components an E1 enzyme, ubiquitin and ATP, and a SCF substrate.

The ubiquitination of the SCF substrate is measured. A chimeric in vitro assay system is provided for measuring CDC53p or CUL1p activity, comprising a CDC4p, CDC34p, and a SKP1p polypeptide, and either a CDC53p or CUL1p polypeptide. In this assay the CDC4p, CDC34p, and SKP1p polypeptide are either a yeast polypeptide or a polypeptide from another species, and at least one of the CDC4p, CDC34p, and SKP1p polypeptides is a yeast polypeptide and at least one of the CDC4p, CDC34p, and SKP1p polypeptides is a polypeptide from another species. A method is further provided for identifying a compound that affects the ability of a CDC4p, a SKP1p, a CDC34p, and a CDC53p or a CUL1p to ubiquitinate a substrate. The method includes contacting a sample comprising a CDC4p, a SKP1p, a CDC34p, and a CDC53p or CUL1p, with the compound under conditions sufficient to allow the components to interact, and adding to these components an E1 enzyme, ubiquitin and ATP, and a substrate for ubiquitination. The ability of the CDC4p, the SKP1p, the CDC34p, and the CDC53p or CUL1p, to ubiquitinate the substrate is measured. A method is also provided of identifying a polypeptide having a function of a CDC4 subunit of SCF. A method is further provided for identifying a polypeptide as a substrate for a ubiquitination reaction.

25 Claims, 0 Drawing figures

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: US 6265174 B1

L6: Entry 1 of 6 File: USPT Jul 24, 2001

US-PAT-NO: 6265174
DOCUMENT-IDENTIFIER: US 6265174 B1

TITLE: Methods and compositions for identifying and modulating ctionprotein-interactions

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Menzel; Rolf	Yardley	PA	N/A	N/A
Hsing; Weihong	Wrightstown	PA	N/A	N/A
Taggart; Pamela A.	Plainsboro	NJ	N/A	N/A

US-CL-CURRENT: 435/7.2; 435/69.7, 435/7.1, 530/350, 536/23.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6232081 B1

L6: Entry 2 of 6 File: USPT May 15, 2001

US-PAT-NO: 6232081
DOCUMENT-IDENTIFIER: US 6232081 B1

TITLE: Method for the detection of NF-.kappa.B regulatory factors

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harper; Jeffrey Wade	Sugarland	TX	N/A	N/A
Elledge; Stephen J.	Houston	TX	N/A	N/A
Winston; Jeffrey T.	Sugar Land	TX	N/A	N/A

US-CL-CURRENT: 435/7.1; 435/7.2, 436/501, 436/516, 436/536

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6165731 A

L6: Entry 3 of 6 File: USPT Dec 26, 2000

US-PAT-NO: 6165731
DOCUMENT-IDENTIFIER: US 6165731 A

TITLE: Assay for the ubiquitination-promoting activity of human proteins

DATE-ISSUED: December 26, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Deshaies; Raymond	Claremont	CA	N/A	N/A
Lyapina; Svetlana	South Pasadena	CA	N/A	N/A
Correll; Craig C.	Pasadena	CA	N/A	N/A

US-CL-CURRENT: 435/7.1; 424/141.1, 435/193, 435/252.3, 435/6, 435/7.4, 435/7.9, 530/387.9, 536/23.2, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 4. Document ID: US 6087153 A

L6: Entry 4 of 6 File: USPT Jul 11, 2000

US-PAT-NO: 6087153
DOCUMENT-IDENTIFIER: US 6087153 A

TITLE: Sel-10 and uses thereof

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Greenwald; Iva	New York	NY	N/A	N/A
Hubbard; E. Jane	Bronx	NY	N/A	N/A

US-CL-CURRENT: 435/252.33; 435/320.1, 530/350, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6037148 A

L6: Entry 5 of 6 File: USPT Mar 14, 2000

US-PAT-NO: 6037148
DOCUMENT-IDENTIFIER: US 6037148 A

TITLE: MTBX protein and nucleic acid molecules and uses therefor

DATE-ISSUED: March 14, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Khodadoust; Mehran	Chestnut Hill	MA	N/A	N/A

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/325, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 6. Document ID: US 5851808 A

L6: Entry 6 of 6 File: USPT Dec 22, 1998

US-PAT-NO: 5851808
DOCUMENT-IDENTIFIER: US 5851808 A

TITLE: Rapid subcloning using site-specific recombination

DATE-ISSUED: December 22, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elledge; Stephen J.	Houston	TX	N/A	N/A
Liu; Qinghua	Houston	TX	N/A	N/A

US-CL-CURRENT: 435/91.4; 435/320.1, 435/91.41, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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L6: Entry 2 of 6 / File: USPT May 15, 2001

US-PAT-NO: 6232081
DOCUMENT-IDENTIFIER: US 6232081 B1

TITLE: Method for the detection of NF-.kappa.B regulatory factors

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harper; Jeffrey Wade	Sugarland	TX	N/A	N/A
Elledge; Stephen J.	Houston	TX	N/A	N/A
Winston; Jeffrey T.	Sugar Land	TX	N/A	N/A

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Baylor College of Medicine	Houston	TX	N/A	N/A	02

APPL-NO: 9/ 172841
DATE FILED: October 15, 1998

PARENT-CASE:
This application is a Continuation-in-Part Application of U.S. pat. appl. Ser. No. 08/951,621, filed Oct. 16, 1997, pending, which is hereby incorporated herein by reference in its entirety.

INT-CL: [7] G01N 33/53, G01N 33/536, G01N 33/561, G01N 33/566
US-CL-ISSUED: 435/7.1; 435/7.2, 463/501, 463/516, 463/536
US-CL-CURRENT: 435/7.1; 435/7.2, 436/501, 436/516, 436/536
FIELD-OF-SEARCH: 435/7.1, 435/7.4, 436/516, 436/536, 436/501

PRIOR-ART-DISCLOSED:

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 652 290	May 1995	EPX	

OTHER PUBLICATIONS

Jiang, J. and Struhl G. Regulation of the hedgehog and wingless signalling pathways by the F-box/WD40-repeat protein Slimb. Nature 39:493-496, Jan. 1998.*
Theodosiou, NA, et al. Slimb coordinates wg and dpp expression in the dorsal-ventral and anterior-porterior axes during limb development. Development 125:3411-3416, 1996.

ART-UNIT: 164
PRIMARY-EXAMINER: Saunders; David
ASSISTANT-EXAMINER: Tung; Mary Beth
ATTY-AGENT-FIRM: Gardere Wynne Sewell LLP Warren, Jr.; Sanford E. Flores; Edwin S.

ABSTRACT:

The present invention provides compositions and methods for gene identification, as well as drug discovery and assessment. In particular, the present invention provides components of an E3 complex involved in ubiquitination of cell cycle regulators and other proteins, as well as members of a class of proteins that directly function in recognition of ubiquitination targets. The present invention also provides sequences of multiple F-box proteins.

4 Claims, 33 Drawing figures

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L6: Entry 2 of 6 File: USPT May 15, 2001

US-PAT-NO: 6232081
DOCUMENT-IDENTIFIER: US 6232081 B1

TITLE: Method for the detection of NF-.kappa.B regulatory factors

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harper; Jeffrey Wade	Sugarland	TX	N/A	N/A
Elledge; Stephen J.	Houston	TX	N/A	N/A
Winston; Jeffrey T.	Sugar Land	TX	N/A	N/A

US-CL-CURRENT: 435/7.1; 435/7.2, 436/501, 436/516, 436/536

CLAIMS:

What is claimed is:
1. A method for the detection of one or more NF-.kappa.B regulatory factors comprising the steps of:
a) providing a slimb protein, and a sample suspected of containing one or more NF-.kappa.B regulatory factors;
b) exposing said slimb protein to said sample under conditions such that said slimb protein binds to said one or more NF-.kappa.B regulatory factors to form a slimb/regulatory factor complex; and
c) detecting said slimb/regulatory factor complex.
2. The method of claim 1, further comprising the step of observing said slimb/regulatory factor complex for degradation of said one or more NF-.kappa.B regulatory factors.
3. The method of claim 1, further comprising the step of exposing said slimb protein and one or more NF-.kappa.B regulatory factors to an F-box protein antagonist.
4. The method of claim 3, wherein said F-box protein antagonist prevents the formation of said slimb/regulatory factor complex.

determined by those experienced in the art by approaches including, but not limited to two-hybrid library screens, immunoprecipitation analysis followed by immunoblotting with antibodies against candidate targets, peptide mapping, mass spectral analysis, peptide sequencing, and/or by screening lambda based expression libraries with F-box protein probes.

DEPR:
For example, the present invention provides an example whereby a novel E3 ubiquitin ligase complex has been identified using the methods and compositions described herein. In particular, the F-box protein slimb (TRCP), was found to associate with I.kappa.B, providing the potential to screen for factors that regulate the NF-.kappa.B pathway. This has important implications in the regulation and control of cancer and the immune system, among other important physiological effects.

DEPR:
The present invention also finds use in investigating the function and methods of altering protein targets whose abundance is altered in disease. For example, cyclins are frequently overexpressed in cancer cells. Thus, mutations in F-box proteins involved in cyclin destruction will lead to cyclin accumulation; such cyclin accumulation may promote inappropriate cell division characteristic of cancer. The present invention also finds utility in the identification of mutations in F-box genes through various methods, including, but not limited sequence analysis, Southern blot analysis of DNA, etc. Furthermore, the present invention also finds use in assessing alterations in cellular protein abundance due to overexpression of particular F-box proteins. It is contemplated that such alterations are associated with particular diseases. The present invention also finds use in determination of overexpression caused by gene amplification in DNA samples from diseased tissue or individuals through such methods as Southern analysis using a particular F-box gene as probe.

DEPR:
As discussed above, the present invention provides compositions and methods for gene identification and characterization, as well as drug discovery and assessment. In particular, the present invention provides components of an E3 complex involved in ubiquitination of cell cycle regulators and other proteins, as well as members of a class of proteins that directly function in recognition of ubiquitination targets (i.e., F-box proteins). These compositions are involved in protein degradation pathways associated with the eukaryotic cell cycle.

DEPR:
The determination that Cdc4 functions in the recognition and ubiquitination of phosphorylated Sic1 is consistent with a function of F-box proteins being recognition of ubiquitination targets. During the development of the present invention, investigations into whether specific F-box proteins could have broad specificity and interact with multiple targets, or could be relatively restricted in their target specificity, perhaps associating with only a single target, were conducted.

DEPR:
To elucidate the selectivity of F-box proteins, experiments were conducted to determine whether substitution of Cdc4 by another F-box protein (Grr1) could support Sic1 binding and ubiquitination. Grr1 has an F-box near its N-terminus and can interact simultaneously with Skp1 and Cdc53 when co-expressed in insect cells. Gene 10-tagged Grr1 (Grr1.sup.10) was also found to interact simultaneously with Skp1 and Cdc53, when co-expressed in insect cells (See, FIG. 4A). It was found that Grr1 and Cdc4 interact with Skp1/Cdc53 in a mutually exclusive manner. In contrast with Cdc4, however, the Grr1/Cdc53 interaction in insect cells was not enhanced by co-expression of Skp1, although Skp1 assembled with these complexes.

DEPR:
Importantly, Grr1 assembled with Cdc53/Skp1 (i.e., Cdc53/Skp1/Grr1 complex) was unable to associate with phosphorylated Sic1 and did not support ubiquitination of phosphorylated Sic1 complexes in the in vitro system with purified proteins under conditions where Cdc4 readily facilitates Sic1 binding and ubiquitination (See, FIGS. 4B and C). Therefore, the F-box proteins of some embodiments of the present invention display selectivity toward particular targets.

DEPR:
Previous studies have shown that mutations of potential Cdc28 phosphorylation sites in the C-terminal PEST domain in Cln2 increase its stability in vivo (Lanker et al., Science 273:1597-1601 [1996]), and that only the phosphorylated form of Cln2 is associated with Cdc53 in vivo (Willems et al., [1996], supra), implicating this interaction in the Cln destruction pathway. Cdc28 is required for Cln phosphorylation although it has not been determined that the requisite phosphorylation reflects autophosphorylation or phosphorylation by a distinct protein kinase. The finding that Sic1 is recognized by the F-box protein Cdc4, together with a genetic requirement for the F-box protein Grr1 in Cln destruction, led to the next step in the development of the present invention, namely the examination of whether Grr1 functions in recognition of phosphorylated Clns.

DEPR:
To generate Cln proteins for binding reactions, Cln/Gst-Cdc28 complexes were isolated from insect cells. In the presence of ATP, both Cln1 and Cln2 were found to be autophosphorylated, a modification that reduces their electrophoretic mobility (see below). To examine whether Grr1 can associate with phosphorylated Clns and to compare the extent of selectivity of Grr1 and Cdc4 toward Cln binding, anti-Skp1.sup.F immune complexes from cells co-expressing Grr1 or Cdc4 in the presence or absence of Cdc53 were used in binding reactions with .sup.32 P-labeled Cln1 or Cln2 kinase complexes. .sup.32 P-labeled Sic1 was used as a control for Cdc4 binding. Both Cln1 and Cln2 complexes were found to associate with Grr1/Skp1.sup.F /Cdc53 complexes (See, FIG. 5A) with an efficiency of about 40% of the input Cln1 or Cln2 (See, FIG. 5A, lanes 8 and 12) and this association did not require Cdc53 (lane 16). In contrast, about 6% of the input Cln proteins associated with Cdc4/Skp1.sup.F complexes independent of the presence of Cdc53 (lanes 7, 11, and 15), compared with 1% association in the absence of an F-box protein (lanes 6, 10, 14). The extent of selectivity of these F-box proteins for Cln and Sic1 was further reflected by the observation that Cln1 protein present in the phosphorylated Sic1 preparation was selectively enriched in Grr1 complexes (FIG. 5A, lane 4). The presence of all proteins in the binding reaction was confirmed by immunoblotting (FIG. 5B) and the quantities of Cdc4 and Grr1 were comparable, based on Coomassie staining of SDS gels of immune complexes. Thus, Grr1 and Cdc4 display specificity toward physiological substrates.

DEPR:
The present invention contemplates that a large number of proteins contain the F-box, and are thereby implicated in the ubiquitin pathway. The development of the present invention has revealed that F-box proteins directly contact ubiquitination substrates and can display selectivity in recognition of potential targets for ubiquitination, as would be expected of E3 proteins. For example, both Grr1 and Cdc4 assemble into mutually exclusive complexes with Cdc53 and Skp1 (FIG. 4). However, Grr1 does not associate with Sic1, nor does it support Sic1 ubiquitination. In contrast, it was found that Cln proteins efficiently associate with Grr1/Skp1.sup.F complexes and with Cdc4/Skp1.sup.F (although less efficiently) (See e.g., FIG. 5). Although Cdc53 was originally isolated as a Cln2-interacting protein (Willems et al., [1996], supra), the present invention provides evidence that this original interaction was bridged by Grr1 and possibly Cdc4. The Grr1/Cln interaction is of interest in view of the fact that GRR1, CDC53, and SKP1 are required for destruction of Cln proteins, and suggests that Grr1 functions as a component of an E3 for Cln ubiquitination. The absence of Cln ubiquitination by purified Grr1 complexes is likely to indicate the absence of an essential factor(s) or modifications that are not required for Sic1 ubiquitination in vitro, and provides evidence that Cln ubiquitination may be more complex than is Sic1 ubiquitination. Nonetheless, the present invention provides methods, compositions, and models for the development of compounds that interact with the ubiquitination process, and thereby affect protein degradation through any number of routes.

DEPR:
Despite the observation that F-box proteins may show selectivity towards potential substrates, it is unlikely that F-box proteins will be monospecific. For example, in S. pombe, recent genetic data have linked the CDC4 homolog pop+with the ubiquitination of both the CK1 Rum1 and Cdc18, a regulator of DNA replication (Kominami and Toda, Genes Dev., 11:1548-1560 [1997]). In budding yeast, CDC4 has also been implicated in destruction of the Cdc18 homolog Cdc6 (Piatti et al., Genes Dev., 10:1516-1531 [1996]), indicating that it too has

multiple targets. It was also determined that Cdc4 can associate with Clns, albeit less efficiently than with Grr1 (FIG. 5). Of importance is the fact that all of the targets of F-box protein mediated destruction identified to date are central regulators of key events in the cell, including DNA replication, cell cycle progression, and nutritional sensing.

DEPR:
Sic1 destruction is genetically dependent upon Cdc34, Cdc4, Cdc53, and Skp1. During the development of the present invention, it was determined that these proteins are directly involved in the ubiquitination process. As Cdc53 can simultaneously bind the E2 Cdc34 and Skp1, it frictions as an adapter linking the Skp1/F-box protein complex to E2s (FIG. 1). In turn, Skp1 has the ability to link Cdc4 to Cdc53. Cdc4 binds both Skp1 and the ubiquitination substrate Sic1. The interaction of Cdc4 with Skp1 was shown to involve the F-box located in the N-terminus of Cdc4, while the interaction with Sic1 involves Cdc4's C-terminal WD40 repeats (FIG. 2). Skp1 was also shown to be involved in substrate recognition because it enhances the association of Cdc4 with phosphorylated Sic1. Cdc4 was shown to act as a receptor that, in conjunction with Skp1, recruits substrates to the ubiquitination complex. It is contemplated that any of these proteins could also have carrier roles in the transfer of ubiquitin like E6AP (See e.g., Scheffner et al., Cell 75:495-505 [1995]). However, it was determined that mutation of the only conserved cysteine in Skp1 or all 6 cysteines in Cdc53 did not impair complementation of skp1 or cdc53 null mutations, respectively, indicating that these two proteins are unlikely to transfer ubiquitin by a thio-ester intermediate.

DEPR:
A central feature in the recognition of Sic1 and Cln by F-box proteins is the phosphorylation dependent nature of the interaction. Association of Sic1 with Cdc4-containing complexes and subsequent ubiquitination requires Sic1 phosphorylation, as shown in FIGS. 2 and 3. It was also shown that Sic1 phosphorylated by excess Clb5/Cdc28 kinase can be ubiquitinated in vitro (See, FIG. 3E). It is contemplated that the initial generation of Clb5/Cdc28 activity at the G1/S transition could potentially accelerate Sic1 destruction facilitating the sharp and unidirectional change of state characteristic of cell cycle transitions.

DEPR:
While regulating the association of F-box proteins through substrate phosphorylation is an effective method controlling the timing of ubiquitination, it is not necessarily the case that all F-box proteins will recognize their substrates in a phosphorylation dependent manner. Observations made during the development of the present invention indicate that WD-40 and LRR containing F-box proteins can interact with phosphorylated substrates, but approximately half of the known F-box proteins do not have obvious protein interaction motifs. Nonetheless, the present invention provides methods, compositions, and models to determine whether the interaction of these proteins with their targets is regulated by phosphorylation or even involves ubiquitination. The timing of ubiquitination could be controlled by mechanisms unrelated to substrate phosphorylation, such as controlled accessibility of substrates or regulated expression, localization, or modification of the F-box protein, thus providing methods for development of compounds that affect proteolysis.

DEPR:
While the abundance of Cdc4 is not cell cycle regulated, the F-box protein Skp2 displays cell cycle-regulated mRNA abundance which peaks in S-phase, consistent with its association with cyclin A during that phase of the cycle (Zhang et al., [1995], supra). In vivo, association of Grr1 and Skp1 is enhanced in the presence of glucose in a post-translational mechanism.

DEPR:
A large number of proteins contain PEST sequences and in a subset of these proteins, these sequences have been shown to be phosphorylated and to mediate instability. The development of one embodiment of the present invention focused on the role of Skp1 and F-box proteins in assembly of a ubiquitination complexes that recognizes specific phosphorylated proteins. While the particular complex defined by this embodiment of the present invention is unlikely to be responsible for recognition of all PEST-dependent proteolysis substrates, this complex is likely to be the prototype for a diverse set of complexes in higher eukaryotes.